PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
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PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*)	14 JUL 2008
Applicant's or agent's file reference 10589-12-228	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US04/01643	21 January 2004 (21.01.2004)	21 January 2003 (21.01.2003)

International Patent Classification (IPC) or both national classification and IPC

IPC: **C12Q 1/68**(2006.01)
USPC: 506/11,16;435/69.1

Applicant

PTC THERAPEUTICS, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I — Basis of the opinion
- ☐ Box No. II — Priority
- ☐ Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV — Lack of unity of invention
- ☒ Box No. V — Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI — Certain documents cited
- ☐ Box No. VII — Certain defects in the international application
- ☐ Box No. VIII — Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(*b*) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/ US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	02 July 2008 (02.07.2008)	STEPHANIE K. MUMMERT Telephone No. 571-272-0872

Form PCT/ISA/237 (cover sheet) (April 2007)

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Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed

☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of:

a. type of material

☐ a sequence listing

☐ table(s) related to the sequence listing

b. format of material

☐ on paper

☐ in electronic form

c. time of filing/furnishing

☐ contained in the international application as filed.

☐ filed together with the international application in electronic form.

☐ furnished subsequently to this Authority for the purposes of search.

4. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

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Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:
   - ☐ paid additional fees
   - ☐ paid additional fees under protest and, where applicable, the protest fee
   - ☐ paid additional fees under protest but the applicable protest fee was not paid
   - ☒ not paid additional fees
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
   - ☐ complied with
   - ☒ not complied with for the following reasons:

   See the lack of unity section of the International Search Report(Form PCT/ISA/210)

4. Consequently, this opinion has been established in respect of the following parts of the international application:
   - ☐ all parts.
   - ☒ the parts relating to claims Nos. 1-31 and 43

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Box No. V Reasoned statement under Rule 43 *bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims Please See Continuation Sheet	YES
	Claims Please See Continuation Sheet	NO
Inventive step (IS)	Claims Please See Continuation Sheet	YES
	Claims Please See Continuation Sheet	NO
Industrial applicability (IA)	Claims Please See Continuation Sheet	YES
	Claims Please See Continuation Sheet	NO

2. Citations and explanations:

Please See Continuation Sheet

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Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

V.1. Reasoned Statements:
The opinion as to Novelty was positive (Yes)with respect to claims 2, 6, 8, 10-12, 17, 20-21, 23, 27-28 and 30-31
The opinion as to Novelty was negative (No) with respect to claims 1, 3-5, 7, 9, 13-16, 18-19, 22, 24-26, 29 and 43
The opinion as to Inventive Step was positive (Yes)with respect to claims 31
The opinion as to Inventive Step was negative(NO) with respect to claims 1-30 and 43
The opinion as to Industrial Applicability was positive (YES) with respect to claims 1-31 and 43
The opinion as to Industrial Applicability was negative(NO) with respect to claims NONE

V. 2. Citations and Explanations:
Claims 1, 3-5, 7, 9, 13-16, 18-19, 22, 24-26, 29 and 43 lack novelty under PCT Article 33(2) as being anticipated by Hyder et al. (Cancer Research. 2000, vol. 60, p. 3183-3190).

With regard to claim 1, Hyder teaches a method for identifying a compound that modulates untranslated region-dependent expression of a vascular endothelial growth factor (VEGF) gene, said method comprising:
(a) contacting a member of a library of compounds with a cell containing a nucleic acid comprising a reporter gene operably linked to an UTR of the VEGF gene (p. 3185, col. 2, where tandem copies of the 5' and 3' UTR upstream of a promoter linked to a luciferase reporter gene and the cell was contacted with ER-alpha or ER-beta and detected reporter gene expression); and
(b) detecting a reporter protein translated from said reporter gene,
wherein a compound that modulates untranslated region-dependent expression of a VEGF gene is identified if the expression of said reporter gene in the presence of a compound is altered as compared to the expression of said reporter gene in the absence of said compound or the presence of a control (p. 3186, col. 2, where the level of the reporter protein activity is measured in the presence of the 3' UTR, the 5' UTR and without the estrogen compound, See C in Figures 5A and 5B).

With regard to claim 3, Hyder teaches an embodiment of claim 1 or 2, wherein the UTR of the VEGF gene is the 5' untranslated region (5' UTR) of a VEGF gene (p. 3185, col. 2, where tandem copies of the 5' and 3' UTR upstream of a promoter linked to a luciferase reporter gene and the cell was contacted with ER-alpha or ER-beta and detected reporter gene expression).

With regard to claim 4, Hyder teaches an embodiment of claim 3 wherein the 5' UTR of the VEGF gene is operably linked upstream of the reporter gene (p. 3185, col. 2, where tandem copies of the 5' and 3' UTR upstream of a promoter linked to a luciferase reporter gene and the cell was contacted with ER-alpha or ER-beta and detected reporter gene expression).

With regard to claim 5, Hyder teaches an embodiment of claim 1 or 2, wherein the UTR of VEGF gene is the 3' untranslated region (3' UTR) of a VEGF gene (p. 3185, col. 2, where tandem copies of the 5' and 3' UTR upstream of a promoter linked to a luciferase reporter gene and the cell was contacted with ER-alpha or ER-beta and detected reporter gene expression).

With regard to claim 7, Hyder teaches an embodiment of claim 3,wherein the nucleic acid further comprises the 3' UTR of a VEGF gene (p. 3185, col. 2, where tandem copies of the 5' and 3' UTR upstream of a promoter linked to a luciferase reporter gene and the cell was contacted with ER-alpha or ER-beta and detected reporter gene expression).

With regard to claim 9, Hyder teaches an embodiment of claim 1 or 2, wherein the reporter gene further comprises an intron (p. 3185, col. 2, where tandem copies of the 5' and 3' UTR upstream of a promoter linked to a luciferase reporter gene and the cell was contacted with ER-alpha or ER-beta and detected reporter gene expression).

With regard to claim 13, Hyder teaches an embodiment of claim 1 or 2, wherein the reporter gene encodes firefly luciferase, renilla luciferase, click beetle luciferase, green fluorescent protein, yellow fluorescent protein, red fluorescent protein, cyan fluorescent

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In case the space in any of the preceding boxes is not sufficient.

protein, blue fluorescent protein, beta-galactosidase, beta-glucoronidase, beta-lactamase, chloramphenicol acetyltransferase, or alkaline phosphatase (p. 3185, col. 2, where tandem copies of the 5' and 3' UTR upstream of a promoter linked to a luciferase reporter gene and the cell was contacted with ER-alpha or ER-beta and detected reporter gene expression).

With regard to claim 14-16, Hyder teaches an embodiment of claim 1, wherein said cell is stably or transiently transfected with said nucleic acid (p. 3184, where HeLa cells were transfected with plasmids comprising the UTR regions upstream of the Tk promoter and the luciferase reporter gene and with plasmids expressing ER-alpha or ER-beta).

With regard to claim 18, Hyder teaches an embodiment of claim 1, wherein the cell is a human cell, a yeast cell, a mouse cell, a rat cell, a Chinese hamster ovary ("CHO") cell, a MCF-7 cell, a primary cell, or an undifferentiated cancer cell (p. 3184, where the human cells were HeLa cells).

With regard to claim 19, Hyder teaches an embodiment of claim 18 wherein the human cell is a HeLa cell or a 293 cell (p. 3184, where the human cells were HeLa cells).

With regard to claim 22, Hyder teaches an embodiment of claim 1 or 2, wherein the compound is selected from a combinatorial library of compounds comprising peptoids, random biooligomers, diversomers, vinylogous polypeptides, nonpeptidal peptidomimetics, oligocarbamates, peptidyl phosphonates, peptide nucleic acid libraries, antibody libraries, carbohydrate libraries, and small organic molecule libraries (p. 3184, 'materials and methods' where the hormones were obtained separately).

With regard to claim 24-26, Hyder teaches an embodiment of claim 1, wherein the step of contacting a library of compounds with a cell is in an aqueous solution comprising a buffer and a combination of salts, wherein the solution mimics physiologic conditions and comprises a detergen (p. 3185, col. 2, where tandem copies of the 5' and 3' UTR upstream of a promoter linked to a luciferase reporter gene and the cell was contacted with ER-alpha or ER-beta and detected reporter gene expression).

With regard to claim 29, Hyder teaches an embodiment of claim 1 or 2, wherein the compound directly binds to an RNA transcribed from the VEGF gene (p. 3185, col. 1, where the ER-alpha and ER-beta compounds bind directly to elements in the 5' and 3'VEGF UTRs).

With regard to claim 43, Hyder teaches an embodiment of claim 1 or 2 further comprising determining the specificity of the compound for the VEGF untranslated region (p. 3185, where the specificity of the binding of the estrogen compounds to the VEGF UTR was analyzed).

Claims 2, 10-12, 17, 20-21 and 30 lack an inventive step under PCT Article 33(3) as being obvious over Hyder in view of Levy et al. (Journal of Biological Chemistry, 1998, vol. 273, no. 11, p. 6417-6423). Hyder teaches analysis of elements in the 5' and 3' UTR of the VEGF gene which modulate expression.

With regard to claim 2, Hyder teaches a method for identifying a compound that modulates untranslated region-dependent expression of a VEGF gene, said method comprising:
(a) contacting a member of a library of compounds with a translation mixture and a nucleic acid comprising a reporter gene operably linked to an UTR of the VEGF gene (p. 3185, col. 2, where tandem copies of the 5' and 3' UTR upstream of a promoter linked to a luciferase reporter gene and the cell was contacted with ER-alpha or ER-beta and detected reporter gene expression); and
(b) detecting the expression of said reporter gene, wherein a compound that modulates untranslated region-dependent expression of a VEGF gene is identified if the expression of said reporter gene in the presence of a compound is altered as compared to the expression of said reporter gene in the absence of said compound or the presence of a control (p. 3186, col. 2, where the level of the reporter protein activity is measured in the presence of the 3' UTR, the 5' UTR and without the estrogen compound, See C in Figures 5A and 5B).

Regarding claim 2, Hyder does not teach analysis in a cell free system.

With regard to claim 2, Levy teaches analysis in a cell free system (p. 6417, 'preparation of S-100 extracts and in vitro RNA Degradation Assays with HuR and HuR antiserum).

With regard to claim 10, Levy teaches an embodiment of claim 1 or 2, wherein the UTR of the VEGF gene comprises an iron response element ("IRE"), internal ribosome entry site ("IRES"), upstream open reading frame ("uORF"), or AU-rich element ("ARE") (Figure 1, where the VEGF, regulatory segment comprises an AU rich region, highlighted in the inset of the figure; p. 6417, col. 2, where it is also noted that the protein binds to an AU-rich element in the VEGF 3' UTR).

With regard to claim 11, Levy teaches an embodiment of claim 1 or 2, wherein the nucleic acid is further polyadenylated at the 3' end (Figure 1, where the nucleic acid is polyadenylated).

With regard to claim 12, Levy teaches an embodiment of claim 1 or 2, wherein the nucleic acid is not capped at the 5' end (Figure 1, where the nucleic acid is not capped at the 5' end).

With regard to claim 17, Levy teaches an embodiment of claim 1 or 2 further comprising measuring the effect of said compound on the expression of the VEGF gene (Figure 5 and 6, where the Western blot shows expression analysis of the VEGF gene).

With regard to claim 20, Levy teaches an embodiment of claim 3, wherein the cell-free translation mixture is a cell extract (p. 6418, col. 2, where cell free extracts from 293T clones were analyzed with HuR affinity purified antiserum).

With regard to claim 21, Levy teaches an embodiment of claim 20, wherein the cell extract is derived from is a human cell, a yeast cell, a mouse cell, a rat cell, a Chinese hamster ovary ("CHO") cell, a Xenopus oocyte, a MCF-7 cell, a primary cell, an undifferentiated cancer cell, a reticulocyte, or a rye embryo (p. 6418, col. 2, where cell free extracts from 293T clones were analyzed with HuR affinity purified antiserum).

With regard to claim 30, Levy teaches an embodiment of claim 1 or 2, wherein the compound binds to one or more proteins that modulate untranslated region-dependent expression of the VEGF gene (Figure 1, where the VEGF, regulatory segment comprises an AU rich region, highlighted in the inset of the figure; p. 6417, col. 2, where it is also noted that the protein binds to an AU-rich element in the VEGF 3' UTR).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have applied the additional VEGF targets to the reporter gene construct format described by Hyder. Levy teaches an analysis of the hypoxic stabilization

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In case the space in any of the preceding boxes is not sufficient.

of VEGF in the presence of an RNA binding protein, HuR, however, the inclusion of this format in the analysis of the control of the hypoxic stabilization, including the analysis of binding sites for the HuR protein would mesh well with the techniques described generally by Hyder.

Claims 6 and 8 lack an inventive step under PCT Article 33(3) as being obvious over Hyder in view of Iida et al. (Life Sciences, 2002, vol. 71, p. 1607-1614). Hyder teaches analysis of elements in the 5' and 3' UTR of the VEGF gene which modulate expression.

Hyder does not teach the inclusion of the 3' UTR operably linked downstream of the reporter gene.

With regard to claim 6 and 8, Iida teaches wherein the 3' UTR of the VEGF gene is operably linked downstream of the reporter gene (Figure 1, where the 5' UTR is placed upstream of the reporter gene and the 3' UTR is placed downstream of the reporter gene).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to vary the location of the 3' UTR relative to the reporter gene in the constructs. As taught by Iida, "we examined the role of the 5' UTR and 3' UTR of VEGF gene in glucose deprived conditions using luciferase assay system. 5' UTR containing reporter vector did not show increase of activity in glucose deprived conditions in contrast to the result in oxygen deprived conditions. Both 5' UTR and 3' UTR containing vector demonstrated significant increase of activity in glucose deficient conditions compared with 5'UTR containing vector" (Abstract). Therefore, it would have been prima facie obvious to one of ordinary skill in the art to have modified Hyder to include constructs with the 3' and 5' UTR in their physiological locations relative to the reporter gene in the construct.

Claims 23, 27 and 28 lack an inventive step under PCT Article 33(3) as being obvious over Hyder in view of Cho et al. (Expert Opin. Ther Targets, 2002, vol. 6, no. 6, p. 679-689). Hyder teaches analysis of elements in the 5' and 3' UTR of the VEGF gene which modulate expression.

Hyder does not teach small organic molecule libraries.

With regard to claim 23, Cho teaches an embodiment of claim 22, wherein the small organic molecule libraries are libraries of benzodiazepines, isoprenoids, thiazolidinones, metathiazanones, pyrrolidines, morpholino compounds, or diazepindiones (Table 2, where a variety of compounds useful in treatment are listed).

With regard to claim 27, Cho teaches an embodiment of claim 1 or 2 further-comprising (c) determining the structure of the compound that modulates untranslated region-dependent expression of the VEGF gene (Figure 8, where in a library where the small molecule comprises a protein, the structure can be determined using mass spectrometry).

With regard to claim 28, Cho teaches an embodiment of claim 27, wherein the structure of the compound is determined by mass spectroscopy, NMR, vibrational spectroscopy, or X-ray crystallography (Figure 8, where in a library where the small molecule comprises a protein, the structure can be determined using mass spectrometry).

It would have been prima facie obvious to one of ordinary skill to include a rationally designed target library in the screening for compounds which modulate expression of VEGF, particularly as controlled or mediated by the 5' or 3' UTR of the VEGF gene. Cho teaches "proteomics analyzes differentially regulated proteins, elucidates protein structure and function, and indentifies interacting partners" (p. 684). Cho also teaches "the most common method in proteome analysis is to perform a 2D gel electrophoresis (2-DE) on a protein sample preparation isolated from a defined set of conditions (i.e. normal versus diseased and control versus drug-treated). Protein bands of interest are digested and identified using mass spectrometry (See Figure 8)" (p. 686). Therefore, it would have been obvious to one of ordinary skill to include a rationally designed target library in the screening for compounds which modulate expression of VEGF, particularly as controlled or mediated by the 5' or 3' UTR of the VEGF gene

Claim 31 meets the criteria set out in PCT Article 33(2)-(3) because the prior art does not teach or reasonably suggest a compound which disrupts an interaction between the 5' UTR and the 3' UTR of the VEGF gene. The closest art of record teaches elements which bind to either the 5' or 3' UTR of the VEGF gene and even elements that include binding sites in both; however there was no apparent evidence of a compound which disrupted an interaction between the 5' and 3' UTR of the VEGF gene.

Claims 1-31 and 43 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.